```
FILE 'CAPLUS' ENTERED AT 14:13:42 ON 01 MAY 1997

=> S RESTRICTION(W)(ENDONUCLEASE OR ENZYME);S LIGASE;S L1 AND L2
         54985 RESTRICTION
         15947 ENDONUCLEASE
        453258 ENZYME
L1       14903 RESTRICTION(W)(ENDONUCLEASE OR ENZYME)


L2        3885 LIGASE


L3         278 L1 AND L2

=> S CLON?
L4      172702 CLON?

=> S L3 AND L4
L5         145 L3 AND L4

=> S GENE
L6      384339 GENE

=> S L6 AND L1
L7        7161 L6 AND L1

=> S L6(3A)L1
L8         620 L6(3A)L1

=> S ZF(3W)FN
           441 ZF
          3055 FN
L9           0 ZF(3W)FN

=> S ZF(4A)FN
           441 ZF
          3055 FN
L10          0 ZF(4A)FN

=> S MAMMAL?;S PLANT;S EUKARYOT? OR EUCARYOT?
L11     113636 MAMMAL?


L12     397433 PLANT


         24700 EUKARYOT?
           771 EUCARYOT?
L13      25256 EUKARYOT? OR EUCARYOT?

=> S L8 AND L11;S L8 AND L12;S L8 AND L13
L14        18 L8 AND L11


L15        20 L8 AND L12


L16        12 L8 AND L13

=> S L14,L15,L16
L17        46 (L14 OR L15 OR L16)

=> D 1-46 CBIB ABS

L17  ANSWER 1 OF 46  CAPLUS  COPYRIGHT 1997 ACS
1997:56255  Document No. 126:71204  Retrovirus vectors derived from
     avian sarcoma leukosis virus permitting transfer of genes into
       ***mammalian***  cells and therapeutic uses thereof.  Barsov,
     Eugene; Hughes, Stephen H. (United States Dept. of Health and Human
```

Services, USA; Barsov, Eugene; Hughes, Stephen H.). PCT Int. Appl. WO 9637625 A1 961128, 65 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 96-US7370 960522. PRIORITY: US 95-445462 950522.

AB Recombinant avian sarcoma leukosis virus (ASLV)-derived retrovirus vectors having an expanded host range are described. The host range is expanded by the replacement of the ASLV envelope gene by an envelope gene from a virus capable of infecting both ***mammalian*** and avian cells. The resulting recombinant ASLV-derived retroviral vectors can replicate efficiently in avian cells, infect both avian and ***mammalian*** cells in high titer, and are replication-defective in ***mammalian*** cells. Thus, they are quite safe and advantageous for use in gene therapy and vaccines. An example is a plasmid vector comprising ASLV LTR, gag, and pol regions, murine leukemia virus ***gene*** env, and a ***restriction*** ***endonuclease*** site for insertion of a gene of interest.

L17 ANSWER 2 OF 46 CAPLUS COPYRIGHT 1997 ACS

1997:38431 Document No. 126:208103 Nucleotide structure and characterization of the murine blood coagulation factor VII gene. Idusogie, Esohe; Rosen, Elliot D.; Carmeliet, Peter; Collen, Desire; Castellino, Francis J. (Department Chemistry Biochemistry, University Notre Dame, Notre Dame, IN, 46556, USA). Thromb. Haemostasis, 76(6), 957-964 (English) 1996. CODEN: THHADQ. ISSN: 0340-6245.

AB The gene encoding murine coagulation factor VII (fVII) was cloned. Seven introns and 8 exons are present, with the introns positioned as splice junctions between the major domain units of the protein. A total of 1,1748 bp of the gene was sequenced, and included 1077 bp of a 5'-flanking region, in which several high probability binding sites for liver transcription factors were present, as well as a CCAAT sequence and possible GC boxes. Primer extension anal. revealed that the major transcription start site was positioned only 9 residues upstream of the ATG initiation codon, thus providing a very short 5'-untranslated region of the gene. The sequence of the CAP site in the murine fVII gene matched exactly the consensus ***eukaryotic*** sequence. A total of 1484 bp of 3'-flanking nucleotides included a probable polyadenylation site (ATTAAA) and an appropriately positioned downstream consensus sequence (AGTGTTTC) for the efficient formation of a 3' terminus of mRNA. These results indicate that all elements are present for liver-based transcription of the gene for murine factor VII. The sequence and ***restriction*** ***endonuclease*** map of this ***gene*** will facilitate construction of fVII deficient mice and mice contg. mutant fVII genes.

L17 ANSWER 3 OF 46 CAPLUS COPYRIGHT 1997 ACS

1997:1366 Document No. 126:55569 Gene cassettes for directional insertion at the SfiI cleavage site in the SV40 replication origin of ***mammalian*** expression vectors. Asselbergs, Fred A. M.; Gandor, Christine; Widmer, Roland (Pharma Res. Dep., CIBA-GEIGY Ltd., Basel, CH-4002, Switz.). Anal. Biochem., 243(2), 285-288 (English) 1996. CODEN: ANBCA2. ISSN: 0003-2697.

AB Expression cassettes and selection markers flanked by SV40 virus origin-compatible SfiI sites are described along with their application for rapid prodn. of recombinant ***mammalian*** cell lines expressing large amts. of heterologous proteins. The pSF vector series contains plasmid vectors with dominant selection genes on the SfiI cassette including dhfr, genes for resistance to the antibiotics geneticin, hygromycin B, mycophenolic acid, and puromycin, as well as the dominant metabolic selection markers trpB and hisD. The cassettes can be combined to create vectors with multiple selection genes, thus improving chances of obtaining cell lines with multiple copies of the vector DNA. The performance of

the SfiI-flanked expression modules was detd. using the expression of hybrid plasminogen activator K2tu-PA in CHO cells as an example. Three highly productive cells lines were obtained after screening only a dozen dhfr-minus CHO cell clones, which had been transfected by SfiI cassette fragments ligated in vitro to give a "copolymer" contg. the dhfr and neo genes in addn. to the K2tu-PA gene.

L17 ANSWER 4 OF 46 CAPLUS COPYRIGHT 1997 ACS
1996:708612 Document No. 126:2219 Nucleic acid sequence and affiliation on pLUG10, a novel cadmium resistance plasmid from Staphylococcus lugdunensis. Chaouni, Loubna Ben-Abdallah; Etienne, Jerome; Greenland, Timothy; Vandenesch, Francois (Dep. Recherche Bacteriologie Medicale, Faculte Medecine R. Laennec, Lyon, 69372, Fr.). Plasmid, 36(1), 1-8 (English) 1996. CODEN: PLSMDX. ISSN: 0147-619X.
AB Tolerance of Staphylococcus lugdunensis to relatively high levels of cadmium is mediated by a 3117-bp plasmid, pLUG10. Sequencing reveals three major open reading frames (ORFs) in a single orientation. One ORF encompasses the origin of replication and its predicted product (RepL; 350 amino acids (aa)) shows 70% homol. in its deduced aa sequence with Rep proteins of the pT181 family. A lagging strand conversion signal (palA) very similar to that of class I plasmids is present outside the rep-ori locus. The other two ORFs of 209 and 116 aa show 92./5% homol. between their deduced aa sequences and the CadB and CadX peptides from the pOX6 plasmid of Staphylococcus aureus. The CadX-like peptide is 40% homologous to the S. aureus CadC product. Deletion of the C-terminal cadX ***gene*** by ***restriction*** ***enzyme*** digestion or frame-shift inactivation of the cadB gene reduced, but did not completely abolish, cadmium resistance. The two gene products may act cooperatively to confer cadmium resistance in S. lugdunensis.

L17 ANSWER 5 OF 46 CAPLUS COPYRIGHT 1997 ACS
1996:435815 Document No. 125:133863 Bacterial community fingerprinting of amplified 16S and 16-23S ribosomal DNA ***gene*** sequences and ***restriction*** ***endonuclease*** analysis (ARDRA). Massol-Deya, Arturo A.; Odelson, David A.; Hickey, Robert F.; Tiedje, James M. (Department Biology, University Puerto Rico, Mayaguez, 00681, P. R.). Mol. Microb. Ecol. Man., 3.3.2/1-3.3.2/8. Editor(s): Akkermans, Antoon D. L.; Van Elsas, Jan Dirk; De Bruijn, Frans J. Kluwer: Dordrecht, Neth. (English) 1995. CODEN: 63APA6.
AB The 16S and 23S rRNA genes have been utilized for phylogenetic anal. of both prokaryotic and ***eukaryotic*** organisms. Various methods have been used to analyze these sequences for identification of bacterial genera and species. In one of these methods, the amplified ribosomal gene (rDNA) is subjected to restriction endonuclease digestion; this has been termed ARDRA for (Amplified Ribosomal DNA Restriction Anal.). The resulting restriction fragment pattern is then used as a fingerprint for the identification of bacterial genomes. This work describes the use of ARDRA for analyzing mixed bacterial populations. A pair of universal priming sequences are used for the amplification of either the 16S rRNA loci or the intergenic regions of the 16S and 23S genes. This PCR based method provides a measure of the structure and compn. of microbial communities.

L17 ANSWER 6 OF 46 CAPLUS COPYRIGHT 1997 ACS
1996:131470 Document No. 124:195142 Detection of Tospovirus species by RT-PCR of the N- ***gene*** and ***restriction*** ***enzyme*** digestions of the products. Dewey, R. A.; Semorile, L. C.; Grau, O. (Instituto de Bioquimica y Biologia Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115 (1900), La Plata, Argent.). J. Virol. Methods, 56(1), 19-26 (English) 1996. CODEN: JVMEDH. ISSN: 0166-0934.
AB Tomato spotted wilt is a serious disease that affects several economically important crops. From the epidemiol. point of view and for the development of a successful plan for transgenic resistance plants, the 4 known Tospovirus species must be discriminated at the mol. level. A RT-PCR assay using primers complementary to the N

gene was used to detect and differentiate 14 Argentinian isolates of Tospovirus from different crops and geog. areas. Exts. were reverse transcribed using a thermoresistant reverse transcriptase and PCR reactions were performed for 30 min in a capillar thermocycler. The products were digested with restriction enzymes and 3 of the 4 described species were identified. Addnl., the results were confirmed by DAS-ELISA. The method described here is rapid and reliable.

L17 ANSWER 7 OF 46 CAPLUS COPYRIGHT 1997 ACS
1995:1000726 Document No. 124:77757 A novel vaccinia virus expression system allowing construction of recombinants without the need for selection markers, plasmids and bacterial hosts. Pfleiderer, M.; Falkner, F. G.; Dorner, F. (Biomed. Res. Cent., Immuno AG, Orth/Donau, 2304/15, Austria). J. Gen. Virol., 76(12), 2957-62 (English) 1995. CODEN: JGVIAY. ISSN: 0022-1317.
AB Vaccinia virus is one of the most widely applied expression systems for use in ***eukaryotes*** in mol. biol. Expression of heterologous genes in the vaccinia virus system, however, requires integration of the foreign DNA into the vaccinia virus genome by means of homologous recombination or by direct mol. cloning. In both cases, plasmid vector constructs are required that contain the gene of interest and, usually, a marker gene, both of which are controlled by suitable promoter sequences. In order to simplify the construction of recombinants and to eliminate the need for a marker gene we have developed a modified vaccinia virus genome that allows the direct targeted insertion of DNA fragments downstream of a strong vaccinia virus promoter without any further cloning steps. The gene of interest is amplified by PCR using oligonucleotide primers that provide an SfiI site at the 5' end and an RsrII site at the 3' end of the PCR product. Following digestion with these restriction enzymes, the PCR product is operationally linked to a synthetic early/late promoter within the viral genomic DNA via the unique SfiI/RsrII sites of the modified vaccinia virus genome. Using this approach, intermediate plasmid constructs and bacterial hosts are not required and time consuming screening steps can be omitted, because 90% of the virus progeny carry the foreign DNA.

L17 ANSWER 8 OF 46 CAPLUS COPYRIGHT 1997 ACS
1995:924034 Document No. 124:2244 Human HOXB cluster and the nerve growth factor receptor gene: Comparison with an orthologous chromosomal domain in mouse. Kevin L. Bentley, M. Suzanne Bradshaw, and Frank H. Ruddle (New Haven, CT, 06511, USA). Genomics, 30(1), 18-24 (English) 1995. CODEN: GNMCEP. ISSN: 0888-7543.
AB The structural organization and nucleotide sequence similarit.gamma. of ***mammalian*** Antennapedia-class homeobox genes support the view that the four homeo.beta..omega.x clusters (HOXA, B, C, and D on human chromosomes 7, 17, 12, and 2, resp.) arose through a combination of gene duplication and divergence t.omega. form a cluster, followed b.gamma. several cluster duplications. The duplication events that gave rise to the four clusters appear to have involved chromosomal domains extending well beyond the borders of the clusters in either direction. This evidence arises from the observation that many genes closely linked to the homeobox clusters on different chromosomes show sequence similarity. Here, we present a continuation of phys. mapping studies to det. the extent and organization of the duplicated regions surrounding the four homeobox clusters in human. Southern blots prepd. from pulsed-field gels of human DNA were probed with cloned segments of human HOXB genes and the nerve growth factor receptor (NGFR) gene on chromosome 17q21-q22. Restriction enzyme anal. revealed the close phys. linkage of these genes within 100 kb. Two yeast artificial chromosomes (YACs), 220 and 380 kb in size, were isolated using oligonucleotide primers specific for NGFR. Both YACs contain the entire HOXB cluster. Restriction mapping of the clones indicated that the distance sepg. these loci could not be greater than 50 kb. This result confirms and extends previous information on the proximity of these genes as detd. by genetic linkage anal. and closely parallels the orthologous loci in the mouse.

L17 ANSWER 9 OF 46 CAPLUS COPYRIGHT 1997 ACS
1995:712187 Document No. 123:77181 Preparation of pathogen-resistant transgenic plants. Blundy, Keith Stuart; O'Reilly, David (Advanced Technologies Bridge Ltd., UK). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1098436 A 950208, 17 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 94-106967 940518. PRIORITY: GB 93-10177 930518.

AB The promoter of tobacco gene KNTI which expression is induced by the infection by Meloidogyne javanica is isolated and used for the prepn. of nematode-resistant plants. A recombinant DNA expressing (1) a gene for cell necrosis (e.g. barnase or RNase) under the control of a pathogen-inducible promoter such as the KNTI gene promoter and (2) a gene for an antagonist (e.g. barstar or a RNase inhibitor) from a ***plant*** promoter can be prepd. for the prepn. of pathogen-resistant plants. Plasmid pS21.08 contg. 2 expression cassettes was prepd.: one for the expression of barnase from the KNTI gene promoter and the other for barstar from CaMV 35S promoter. The plasmid was used to prep. nematode-resistant plants.

L17 ANSWER 10 OF 46 CAPLUS COPYRIGHT 1997 ACS
1995:679995 Document No. 123:247773 Cloning and applications of the two/three-base restriction endonuclease R.cntdot.CviJI from IL-3A virus-infected Chlorella. Skowron, Piotr M.; Swaminathan, Neela; McMaster, Karolyn; George, David; Van Etten, James L.; Mead, David A. (Molecular Biology Resources Inc, Milwaukee, WI, 53210, USA). Gene, 157(1/2), 37-41 (English) 1995. CODEN: GENED6. ISSN: 0378-1119.

AB The gene (cviJIR) encoding the two/three-base R.cntdot.CviJI ***eukaryotic*** restriction endonuclease (ENase) from IL-3A virus-infected Chlorella was cloned into Escherichia coli. A high frequency of DNA cleavage by R.cntdot.CviJI required overexpression of the gene encoding the M.cntdot.CviJI methyltransferase prior to cloning the gene for the ENase. Both genes were sequenced and their organization was detd. to be in head-to-tail order. The open reading frame coding for R.cntdot.CviJI can potentially translate a 41.4-kDa protein; however, in the E. coli host, a truncated version of the enzyme is produced (32.5 kDa). The recombinant ENase does not exhibit ATP-induced star activity (R.cntdot.CviJI cleaves at RGCY, whereas R.cntdot.CviJI also cleaves at RGCR and YGCY, but not at YGCR), as is characteristic for native R.cntdot.CviJI. The very high frequency of DNA cleavage by R.cntdot.CviJI was exploited in the development of a quasi-random shotgun library method. R.cntdot.CviJI-generated oligodeoxyribonucleotides were applied to improve certain mol. biol. applications, i.e., DNA labeling, detection, high-resoln. restriction mapping, amplification, and epitope mapping.

L17 ANSWER 11 OF 46 CAPLUS COPYRIGHT 1997 ACS
1995:284867 Document No. 122:124385 Tagged mutations at the Tox1 locus of Cochliobolus heterostrophus by restriction enzyme-mediated integration. Lu, Shunwen; Lyongholm, Linda; Yang, Ge; Bronson, Charlotte; Yoder, O. C.; Turgeon, B. Gillian (Dep. Plant Pathol., Cornell Univ., Ithaca, NY, 14853, USA). Proc. Natl. Acad. Sci. U. S. A., 91(26), 12649-53 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.

AB The authors have used the restriction enzyme-mediated integration insertional mutagenesis procedure to tag the Tox1 locus in the filamentous Ascomycete Cochliobolus heterostrophus. Mutations at other, unselected, loci were also identified and a high proportion (30-50%) of them were tagged. This procedure may be of general utility for simultaneously mutating and tagging genes in fungi and in other ***eukaryotes*** . The Tox1 locus of C. heterostrophus has been defined by Mendelian anal. as a single genetic element that controls prodn. of T toxin, a linear polyketide involved in virulence of the fungus to its host ***plant*** , corn. To tag Tox1, protoplasts of a Tox1+ (T-toxin producing) strain were transformed with a linearized, nonhomologous plasmid along with an excess of the restriction enzyme used to linearize the plasmid. Of 1310 transformants recovered, two produced no detectable T toxin in

culture or on corn plants. In each of these transformants, the Tox- mutation mapped at Tox1, was tagged with the selectable marker (hygB) on the transforming plasmid, and was tightly linked to the other tagged Tox- mutation. The two mutations, however, represent two different points of plasmid insertion at the Tox1 locus.

L17 ANSWER 12 OF 46 CAPLUS COPYRIGHT 1997 ACS
1994:263078 Document No. 120:263078 Process for gene targeting and genome manipulations. Schiestl, Robert H.; Petes, Thomas D.; Kong, Stephanie E. (Genebiomed, Inc., USA). PCT Int. Appl. WO 9323534 A1 931125, 97 pp. DESIGNATED STATES: W: AU, CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 93-US4847 930521. PRIORITY: US 92-887689 920521; US 92-944665 920914.

AB A process for insertional mutagenesis and genome manipulation of yeast, Dictyostelium and ***mammalian*** cells by introducing a recombination substrate is provided. A recombination substrate lacking sequence homol. with the host genome integrates into it in the absence or presence of restriction enzymes use to promote illegitimate recombination. A process for screening a potential toxic material on the illegitimate recombination frequency is also provided. The method is demonstrated by restoration of a URA3+ phenotype to a Saccharomyces strain from which the URA3 gene had been deleted. Cells with the URA3 deletion were transformed with a transformation buffer contg. a BamHI fragment contg. the URA3 ***gene*** and BamHI ***restriction*** ***enzyme*** with a frequency of 5-100 URA3+ transformants/.mu.g DNA. Genetic and phys. characterization of a set of transformants showed one integration event/haploid genome with the integrations unlinked to the URA3 locus. The integration sites were all flanked by BamHI sites with the sequences beyond these sites different.

L17 ANSWER 13 OF 46 CAPLUS COPYRIGHT 1997 ACS
1994:186109 Document No. 120:186109 Chimeric restriction endonuclease. Kim, Yang Gyun; Chandrasegaran, Srinivasan (Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205-2179, USA). Proc. Natl. Acad. Sci. U. S. A., 91(3), 883-7 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.

AB FokI restriction endonuclease recognizes the nonpalindormic pentadeoxyribonucleotide 5'-GGATG-3'.cntdot.5'-CATCC-3' in duplex DNA and cleaves 9 and 13 nt away from the recognition site. Recently, the authors reported the presence of two distinct and separable domains within this enzyme: one for the sequence-specific recognition of DNA (the DNA-binding domain) and the other for the endonuclease activity (the cleavage domain). Here, the authors report the construction of a chimeric restriction endonuclease by linking the Drosophila Ultrabithorax homeodomain to the cleavage domain (FN) of FokI restriction endonuclease. The hybrid enzyme, Ubx-FN, was purified, and its cleavage properties were characterized. The hybrid enzyme shows the same DAN sequence-binding preference as that of Ubx; as expected, it cleaves the DNA away from the recognition site. On the 5'-TTAATGGTT-3' strand the hybrid enzyme cleaves 3 nt away from the recognition site, whereas it cuts the complementary 5'-AACCATTAA-3' strand 8, 9, or 10 nt away from the binding site. Similarly engineered hybrid enzymes could be valuable tools in phys. mapping and sequencing of large ***eukaryotic*** genomes.

L17 ANSWER 14 OF 46 CAPLUS COPYRIGHT 1997 ACS
1994:1676 Document No. 120:1676 Synthesis and expression of a gene for mature small subunit of rice rubisco. Chen, Haibao; Wang, Guoan (Shanghai Inst. Org. Chem., Chin. Acad. Sci., Shanghai, 200032, Peop. Rep. China). Curr. Plant Sci. Biotechnol. Agric., 15(Biotechnology in Agriculture), 160-4 (English) 1993. CODEN: CPBAE2. ISSN: 0924-1949.

AB A designed gene for mature small subunit of rice ribulose bisphosphate carboxylase/oxygenase [Rubisco, EC 4.1.1.39] has been synthesized and expressed in Escherichia coli. The gene, with 396 b.p., was designed to have 20 unique ***restriction***

endonuclease sites throughout the ***gene*** . Synthesis of the gene was carried out by single strand method. After the sequence of the synthetic gene was confirmed, a designed SD sequence for overexpression of the gene in E. coli was inserted upstream from the gene and cloned into an E. coli expression vector pLc2833 which utilizes the left promoter (PL) of phage. E. coli c600[pcI857] cells were transformed by the plasmid and temp.-induced, the small subunit was expressed up to 30% of the total cell proteins. Preliminary purifn. of the products yielded the rice rbcS with 90% homogeneity.

L17 ANSWER 15 OF 46 CAPLUS COPYRIGHT 1997 ACS
1993:421298 Document No. 119:21298 Use of restriction endonucleases to detect and isolate genes from ***mammalian*** cells. Bickmore, Wendy A.; Bird, Adrian P. (MRC Hum. Genet. Unit, West. Gen. Hosp., Edinburgh, EH4 2XU, UK). Methods Enzymol., 216(Recombinant DNA, Pt. G), 224-44 (English) 1992. CODEN: MENZAU. ISSN: 0076-6879.
AB A review and discussion with 36 refs. Unusual sequences (non-methylated CpG islands) at the 5' ends of most ***mammalian*** genes can be located using certain types of restriction endonucleases, thereby facilitating the mapping and cloning of the gene of interest. The factors affecting choice of rate-cutting restriction enzymes for mapping and cloning expts. are discussed, and examples of their use are presented.

L17 ANSWER 16 OF 46 CAPLUS COPYRIGHT 1997 ACS
1993:228447 Document No. 118:228447 Cloning and sequence analysis of a calcium-binding protein gene from a pathogenic strain of Entamoeba histolytica. Prasad, Jayendra; Bhattaharya, Sudha; Bhattacharya, Alok (Sch. Life Sci., Jawaharlal Nehru Univ., New Delhi, 110067, India). Mol. Biochem. Parasitol., 52(1), 137-40 (English) 1992. CODEN: MBIPDP. ISSN: 0166-6851.
AB Although a no. of mols. involved in target cell recognition by E. histolytica have been identified, the mol. mechanism of pathogenesis is yet unclear. Recently, Ca2+ has been implicated in the pathogenesis of amoebiasis. In this study the nucleotide sequence of a (calcium-binding protein) gene from E. histolytica is reported. A cDNA expression library of E. histolytica HM1:IMSS, clone 6 in .lambda.gt11 was immunoscreened with a polyclonal antibody (aEhT.KCG). Four clones were isolated and purified after 4 rounds of immunoscreening and subcloned into the plasmid pTZ18U. Hybridisation anal. showed that all 4 clones represent the same insert of about 300 bp. Sequence detn. of one of the clones revealed a substantial homol. to calmodulin, a CaBP. This cDNA (pCAM, 297 bp) was partial and lacked both the 3'- and 5'-ends of the putative gene. It was used as a probe to screen a genomic library in .lambda.ZAP to obtain the full-length gene. Six genomic clones were purified, of which 2 showed a 2-kb insert contg. the upstream and downstream regions of the ***gene*** (confirmed by ***restriction*** ***enzyme*** digestion and Southern blot anal. One of these clones (pGENCAM) was chosen for further anal. The nucleotide sequence was detd. by the dideoxy chain termination method using double-stranded DNA. The complete gene had an ORF of 402 bp and lacked introns. Computer anal. of the deduced amino acid sequence from the 402-bp ORF through sequence data bank showed striking similarities with all known calmodulins and a few other CaBPs. The E. histolytica sequence contained 4 domains which matched very closely the Ca2+-binding domains of calmodulin and CaBPs from a variety of ***eukaryotes*** . In addn., the 4 putative Ca2+ binding domains (I-IV) of E. histolytica were sepd. from each other by a similar no. of amino acids (24, 27 and 20 amino acids, resp.) to that found in calmodulins from other sources (24, 25 and 24 amino acids, resp.). Despite this striking structural similarity, the amino acid sequence of the E. histolytica gene had a max. homol. of only 35% with other known calmodulins. A similar lack of extensive sequence homol. has been reported for yeast calmodulin. The calcium-binding properties of this CaBP were also studied.

L17 ANSWER 17 OF 46 CAPLUS COPYRIGHT 1997 ACS
1993:184550 Document No. 118:184550 Study on molecular biology of rice stripe virus. II. Synthesis, cloning and expression of coat protein gene. Qiu, Bingsheng; Wang, Jinfang; Tian, Bo; Tien, Po (Inst. Microbiol., Acad. Sin., Beijing, 100080, Peop. Rep. China). Chin. Sci. Bull., 37(13), 1121-5 (English) 1992. CODEN: CSBUEF. ISSN: 1001-6538.

AB A cDNA for rice stripe virus genome was synthesized by using Hg hydroxymethyl-denatured poly(A)-terminated RNA. This cDNA was cloned and expressed in Escherichia coli and the coat protein gene was identified in recombinant clones by hybridization using a viral gene probe. The recombinant ***gene*** was analyzed using ***restriction*** ***endonuclease*** digestion, hydridization, and Northern blot anal.

L17 ANSWER 18 OF 46 CAPLUS COPYRIGHT 1997 ACS
1993:143620 Document No. 118:143620 Restriction enzyme analysis of the chloroplast and nuclear 45 S ribosomal DNA of Allium sections Cepa and Phyllodolon (Alliaceae). Havey, M. J. (Dep. Hortic., Univ. Wisconsin, Madison, WI, 53706, USA). Plant Syst. Evol., 183(1-2), 17-31 (English) 1992. CODEN: ESPFBP. ISSN: 0378-2697.

AB Ests. of the phylogenetic relationships among cultivated and wild Allium species would benefit from identification of mol. characters. Restriction enzyme anal. of the chloroplast DNA (cpDNA) of the bulb onion (Allium cepa), Japanese bunching onion (A. fistulosum), wild Allium species in sect. Cepa and Phyllodolon, and the outgroups A. ampeloprasum and A. tuberosum detected 39 polymorphisms. A. cepa and A. vavilovii were identical for all characters. Cladistic anal. generated three most-parsimonious Wagner trees of 44 steps differing only in a zero-length branch. A. fistulosum and A. altaicum (sect. Phyllodolon) comprised a monophyletic lineage sepd. from the A. cepa and A. vavilovii of sect. Cepa. The unresolved node was composed of A. galanthum, A. roylei, and the lineage contg. A. cepa, A. vavilovii, A. fistulosum, and A. altaicum. The clade contg. A. altaicum, A. cepa, A. fistulosum, A. galanthum, A. roylei, and A. vavilovii remained resolved for strict consensus of Wagner trees of 48 steps or less. A. pskemense and A. oschaninii were increasingly distant. A. oschaninii has been proposed as the progenitor of the bulb onion, but was more closely related to the common progenitor of all species in sect. Cepa and Phyllodolon. Phylogenies estd. from cpDNA characters using Dollo parsimony resulted in a single most-parsimonious tree of 46 steps and agreed with phylogenies based on Wagner parsiomony. Polymorphic restriction enzyme sites in the 45 S ribosomal DNA were not used to est. phylogenies because of uncertain homologies, but are useful for identifying interspecific hybrids. The maternal phylogenies estd. in this study help to distinguish wild Allium species closely related to the bulb onion. Although not in agreement with classifications based on morphol., the phylogenies closely reflected crossability among species in sect. Cepa and Phyllodolon.

L17 ANSWER 19 OF 46 CAPLUS COPYRIGHT 1997 ACS
1992:585841 Document No. 117:185841 Polymerase chain reaction comparison of the gene for strictosidine synthase from ten Rauvolfia species. Bracher, Daniel; Kutchan, Toni M. (Univ. Muenchen, Munich, W-8000/2, Germany). Plant Cell Rep., 11(4), 179-82 (English) 1992. CODEN: PCRPD8. ISSN: 0721-7714.

AB The gene for strictosidine synthase, str1, has been analyzed by the polymerase chain reaction in ten species of Rauvolfia, the origins of which span the tropical belt: R. cambodiana (Indochina), R. canescens (India), R. chinensis (China), R. heterophylla (Central America), R. mannii (West Africa), R. nitida (West Indies), R. praecox (Brasil), R. serpentina (India), R. sumatrana (Indonesia) and R. verticillata (Indochina). ***Restriction*** ***endonuclease*** anal. of the ***gene*** fragments produced with genomic DNA from each of the ten species as template revealed that str1 is highly conserved in the Rauvolfia species investigated. These results suggest that there is a stringent selection pressure on the gene for this key enzyme of indole alkaloid biosynthesis.

L17 ANSWER 20 OF 46 CAPLUS COPYRIGHT 1997 ACS
1992:188743 Document No. 116:188743 Use of Dpn I restriction enzyme to assess newly replicated gene copies in amplifiable vector systems. Brewer, Alison C.; Patient, Roger K. (King's Coll., Univ. London, London, UK). Methods Mol. Biol. (Totowa, N. J.), 7(Gene Transfer Expression Protocols), 405-10 (English) 1991. CODEN: MMBIED.
AB A method for assessing whether replication has occurred in a transient transfection expt. and/or quantifying the extent of that replication is described. This information is important if replication affects gene expression and can provide convincing evidence of successful transfection. Replication can be readily monitored using the methylation-sensitive restriction enzyme DpnI. DpnI will digest only DNA that is bacterially methylated on both strands. Most strains of Escherichia coli used to propagate plasmid DNA contain a site-specific methylase encoded by the dam gene that transfers a Me group to the N7 position of the adenine residues in the sequence 5' GATC 3'. 5' GATC 3' is the recognition sequence of the DpnI restriction enzyme and, provided the DNA to be used in the transfection expt. has been grown up in a dam+ bacterial strain, all the 5' GATC 3' sites will be fully A-methylated and will therefore all be digestible with DpnI. ***Eukaryotic*** cells lack these A methylation enzymes. Thus, if the transfected DNA copies undergo one or more rounds of replication, they will become first hemi- and then unmethylated at the A residues and so refractory to digestion by DpnI. Digestion of transiently transfected DNA by DpnI can therefore be used to quantitate replication in an amplifiable vector system.

L17 ANSWER 21 OF 46 CAPLUS COPYRIGHT 1997 ACS
1991:530920 Document No. 115:130920 Analysis of rice (Oryza sativa L.) genome using pulsed-field gel electrophoresis and rare-cutting restriction endonucleases. Sobral, Bruno W. S.; Honeycutt, Rhonda J.; Atherly, Alan G.; McClelland, Michael (California Inst. Biol. Res., La Jolla, CA, 92037, USA). Plant Mol. Biol. Rep., 8(4), 253-75 (English) 1990. CODEN: PMBRD4. ISSN: 0735-9640.
AB Techniques for the prepn. of intact genomic DNA from Indica and Japonica subspecies of rice were developed, statistical methods were used to det. which restriction endonucleases are rare-cutting, and pulse-field gel electrophoresis (PFE) was used to sep. large fragments of rice DNA. Southern hybridization to blotted rice PFE gels was used to show that the digests were complete.

L17 ANSWER 22 OF 46 CAPLUS COPYRIGHT 1997 ACS
1991:507788 Document No. 115:107788 Two dimentional electrophoresis for screening genomic DNA of higher organism. Mukai, Tsunehiro; Hayashizaki, Yoshihide; Hatada, Izuho; Hirotsune, Shinji (Japan). Jpn. Kokai Tokkyo Koho JP 03053899 A2 910307 Heisei, 8 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 89-190035 890721.
AB The genomic DNA of higher organism is screened by a combination of two dimensional electrophoresis and multiple restriction enzymes. The method comprises restriction of the genomic DNA with first restriction enzyme and labeling of the DNA fragments, first dimensional gel electrophoresis of the labeled DNA fragments, again restriction of the labeled DNA fragments (on gel) with second restriction enzyme, and second dimensional gel electrophoresis of the further restricted DNA fragments. Screening of Drosophila melanogaster genomic DNA was exemplified.

L17 ANSWER 23 OF 46 CAPLUS COPYRIGHT 1997 ACS
1991:486620 Document No. 115:86620 Efficient construction of ***plant*** genomic libraries requires the use of mcr host strains and packaging mixes. Graham, Michael W.; Doherty, Judith P.; Woodcock, David M. (Calgene Pacific Pty. Ltd., Collingwood, 3066, Australia). Plant Mol. Biol. Rep., 8(1), 18-27 (English) 1990. CODEN: PMBRD4. ISSN: 0735-9640.
AB It has recently become apparent that many strains of Escherichia coli contain nucleases encoded by the mcrA and mcrB loci that recognize the modified base 5-methylcytosine in DNA. ***Plant***

DNAs have particularly high levels of this modification, and the activity of these 5-methylcytosine-specific nucleases is particularly relevant to cloning ***plant*** genomic DNAs. For prepg. libraries in a .lambda. replacement vector, the use of suitable mcr-hosts and mcr-packaging mixes can increase the efficiency of cloning of ***plant*** genomic DNAs by .gtoreq.2-fold. The activity of the mcr nucleases is probably a significant source of bias in the representation of sequences in ***plant*** genomic libraries.

L17 ANSWER 24 OF 46 CAPLUS COPYRIGHT 1997 ACS
1991:423311 Document No. 115:23311 Reduced chloramphenicol acetyltransferase activity observed with vectors containing an upstream SphI recognition sequence. Alam, J.; Yu, N.; Irias, S.; Cook, J. L.; Vig, E. (Dep. Mol. Genet., Alton Ochsner Med. Found., New Orleans, LA, 70121, USA). BioTechniques, 10(4), 422, 424-5 (English) 1991. CODEN: BTNQDO. ISSN: 0736-6205.
AB Chloramphenicol acetyltransferase (CAT) is the most commonly used receptor gene for studying the regulation of ***mammalian*** gene transcription. Some of the currently available CAT vectors contain the recognition sequence for the restriction endonuclease SphI within the multiple cloning site. This sequence introduces an ATG triplet that is out of frame with the initiation codon of the CAT gene. Transient expression of CAT fusion genes, constructed using 3 different cellular promoters, demonstrates that the presence of the upstream AUG triplet in the CAT transcript reduces CAT activity, presumably by interfering with the translation of the coding sequence. Deletion of the SphI site from each of the plasmids increased CAT activity between 4-fold and 5-fold. Thus, upstream, out-of-frame ATG triplets must be avoided in order to achieve max. expression of the reporter gene.

L17 ANSWER 25 OF 46 CAPLUS COPYRIGHT 1997 ACS
1991:200974 Document No. 114:200974 Peculiarity of ***plant*** DNA cloning. Kozlovskii, Yu. E.; Shiyan, A. N. (Sci. Res. Inst. Peltry-Ware Rabbit Breed., Rodniki, 140143, USSR). Mol. Biol. (Moscow), 24(3), 859-63 (Russian) 1990. CODEN: MOBIBO. ISSN: 0026-8984.
AB The efficiency of pea (Pisum sativum) DNA cloning into phage vector EMBL4 has been tested on different strains of Escherichia coli. Recombinant phage prodn. was very low in a no. of E. coli strains used routinely for gene bank construction. However, the efficiency of prodn. of recombinant phage was higher in E. coli strain K802. Its proposed that this effect is due to the absence of the McrB system for restriction of DNA methylated at cytosine residues in strain K802. The efficiency of pea DNA cloning was not enhanced in strain C600 in which only the McrA restriction system was absent.

L17 ANSWER 26 OF 46 CAPLUS COPYRIGHT 1997 ACS
1990:152986 Document No. 112:152986 Co-transfer of restriction endonucleases and plasmid DNA into ***mammalian*** cells by electroporation: effects on stable transformation. Yorifugi, Tohru; Mikawa, Haruki (Fac. Med., Kyoto Univ., Kyoto, 606, Japan). Mutat. Res., 243(2), 121-6 (English) 1990. CODEN: MUREAV. ISSN: 0027-5107.
AB Cloned HSV thymidine kinase gene and restriction endonucleases, HindIII, XbaI, or XhoI, were cotransfected into thymidine kinase-deficient mouse Ltk- cells by electroporation. Stimulation of the transformation efficiency was obsd. with HindIII or XbaI, whereas little effect was obsd. with XhoI. The stimulation was obsd. for various forms of exogenous DNA (linear or circular plasmid DNA and single-stranded phage DNA) and correlated with the no. of strand breaks in the host-cell DNA. These results suggest that the presence of DNA double-strand breaks stimulates the integration of exogenous DNA into host cell genomes.

L17 ANSWER 27 OF 46 CAPLUS COPYRIGHT 1997 ACS
1990:51149 Document No. 112:51149 Two classes of observed frequency for rare-cutter sites in CpG islands. Bird, Adrian P. (Res. Inst.

Mol. Pathol., Vienna, 1030, Austria). Nucleic Acids Res., 17(22), 9485 (English) 1989. CODEN: NARHAD. ISSN: 0305-1048.

AB The finding that nonmethylated sites for rare-cutting restriction enzymes are concd. in CpG islands has helped in the construction of long range maps of ***mammalian*** DNA and in the search for genes. The expected frequencies of rare-cutter sites in CpG islands have been calcd. previously. This report compares those ests. with the obsd. frequencies in CpG islands at 19 human genes taken from the Genbank database (fola1, fos, H4, hba4, his10g, hmg14, hsp27, int1, met2, metia, mha2, mhdcb, mycc, rash, rbp1, rps14, sisg5, tka, tpi). Islands were identified by CpG/GpC ratios. Some 30.45 kb of CpG island DNA was analyzed. Only 8 islands could be considered complete, and their av. length was 1.1 kb. Rarecutters were divided into 2 classes: [a] enzymes recognizing G+C-only sites which cut at close to the expected frequency in CpG islands (NotI in fact cuts 3 times more often then predicted); [b] enzymes recognizing sites with 2 CpGs plus A and T which cut up to ten times less frequently than expected. Most CpG islands have sites for class [a] enzymes, whereas inter-island sites are rare in comparison and blocked by methylation. The very low frequency of class [b] sites (10-40 times less than class [a] six-cutters) means that cuts in genomic DNA are more likely to occur at rare nonmethylated sites between islands than within the islands themselves. Thus, cleavage will often be partial, and fragments will be larger than those given by class [a] enzymes. The results imply that the choice between these 2 groups of enzymes will significantly influence the kind of data generated by long range mapping expts.

L17 ANSWER 28 OF 46 CAPLUS COPYRIGHT 1997 ACS

1990:49857 Document No. 112:49857 Genome analysis of rainbow trout and sturgeon with restriction enzymes and hybridization with a ZFY gene derived probe to identify sex. Ferreiro, Carmen; Medrano, Juan Fernando; Gall, Graham A. E. (Dep. Anim. Sci., Univ. California, Davis, CA, 95616, USA). Aquaculture, 81(3-4), 245-51 (English) 1989. CODEN: AQCLAL. ISSN: 0044-8486.

AB An initial evaluation of mol. screening methods to sep. male and female rainbow trout and sturgeon was performed. High mol. wt. genomic DNA was obtained from red blood cells and cut with various restriction enzymes. Digests were examd. for unique repetitive DNA sequences assocd. with the heterogametic chromosome. For all the enzymes tested, no characteristic banding patterns were obsd. for either of the 2 sexes. As in other ***eukaryotes*** , significant nos. of repetitive nucleotide regions were detected in the organization of the trout and sturgeon genome. A specific DNA probe from humans (pDP1007) that detects a single copy sequence on the ***mammalian*** Y-chromosome was evaluated for its specificity in discriminating male from female individuals. The DNA segment of probe pDP1007 had homol. with genomic DNA of trout and sturgeon, but no differences were obsd. between the male and female pattern of hybridization. The exptl. approach applied in the study is discussed in relation to sex detg. mechanisms in fish.

L17 ANSWER 29 OF 46 CAPLUS COPYRIGHT 1997 ACS

1990:1867 Document No. 112:1867 Structural difference of the cytochrome oxidase subunit II genes between normal and male sterile cytoplasm in sugar beet. Harada, Takeo; Mikami, Tetsuo; Kinoshita, Yoshiro (Hokkaido Prefect. Cent. Agric. Exp. Stn., Naganuma, 069, Japan). Tensai Kenkyu Kaiho, 30, 118-22 (Japanese) 1988. CODEN: TKKADS.

AB The trait of cytoplasmic male sterility (CMS) has been used extensively in the com. prodn. of hybrid seed as a means of preventing self-fertilization. The prevailing evidence in ***plant*** species indicates that cytoplasmic male sterility is coded for in the mitochondrion and not in the chloroplast. In order to investigate the mol. basis of CMS in sugar beet, the authors isolated and sequenced the mitochondrial DNA fragment of fertile (N) cytoplasm which contains the gene for subunit I of cytochrome c oxidase (coxI). Computer translation of DNA sequences upstream from the coxI gene revealed the 3' exon of gene coxII. Using this cloned

sugar beet coxII gene as a hybridization probe, it was shown that sugar beet coxII has an 1.2 kbp intron corresponding to the intron found in rice and carrot. The hybridization pattern indicated that N mitochondrial genome contains single copies of the coxII gene, whereas sterile(S) mtDNA has two copies of the gene. However, regarding the 3' flanking region of coxII, one ***gene*** has the same ***restriction*** ***enzyme*** map as N cytoplasm, but the other coxII gene has not. Hybridization expts. suggested that a recombination event produces an unique extra truncated coxII gene in S cytoplasm. These results suggest that this coxII gene may cause CMS trait in sugar beet.

L17 ANSWER 30 OF 46 CAPLUS COPYRIGHT 1997 ACS
1989:626359 Document No. 111:226359 Organization of the human corticosteroid-binding globulin gene and analysis of its 5'-flanking region. Underhill, D. Alan; Hammond, Geoffrey L. (Dep. Obstet. Gynecol., Univ. West. Ontario, London, ON, N6A 4G5, Can.). Mol. Endocrinol., 3(9), 1448-54 (English) 1989. CODEN: MOENEN. ISSN: 0888-8809.
AB The structure of the human corticosteroid binding globulin (CBG) ***gene*** was detd., and ***restriction*** ***endonuclease*** maps of human placental DNA and cloned genomic DNA indicate that CBG is encoded by a single gene. The transcription unit for hepatic CBG mRNA comprises 5 exons distributed over .apprx.19 kilobases (kb), and nuclease protection and primer extension studies using human liver RNA demonstrate that the first exon spans 70 base pairs (bp). Typical of many ***eukaryotic*** promoters, sequences that resemble TATA and CAAT-box motifs are centered 28 bp and 73 bp upstream from the origin of transcription, resp. In addn., 6 highly conserved sequence elements, responsible for efficient, liver-specific expression of the mouse albumin gene, are located within the first 200 bp of the 5'-flanking region. Further anal. of a region (500 bp) immediately 5' of the transcription start site, however, failed to reveal sequences that might correspond to known steroid hormone response elements. When compared to other serine protease inhibitor genes, the organization of the human CBG gene is most closely related to the human .alpha.1-proteinase inhibitor and .alpha.1-antichymotrypsin genes. It would therefore appear that these proteins are derived from a common ancestral gene, and this supports the concept that they may be functionally related.

L17 ANSWER 31 OF 46 CAPLUS COPYRIGHT 1997 ACS
1989:420058 Document No. 111:20058 Evolution of type II DNA methyltransferases. A gene duplication model. Lauster, Roland (Max-Planck-Inst. Mol. Genet., Berlin, D-1000/33, Fed. Rep. Ger.). J. Mol. Biol., 206(2), 313-21 (English) 1989. CODEN: JMOBAK. ISSN: 0022-2836.
AB On the basis of consensus sequences, which were previously defined for 2 groups of closely related cytosine-specific and adenine-specific DNA methyltransferases, homologies can be detected that indicate a common origin for these proteins. Intramol. comparisons of several of these enzymes reveal homol. relationships, which suggests that gene duplication is a phylogenetic principle in the evolution of the methyltransferases. One or 2 duplications of an ancestral gene encoding a 12,000-16,000 Mr protein, followed by divergent evolution, may have led to very different protein structures and could explain the differences in amino acid sequences, mol. wts., and biochem. properties. Intermol. and intramol. homologies were also recognized in type II restriction endonucleases, suggesting a very similar evolutionary pathway.

L17 ANSWER 32 OF 46 CAPLUS COPYRIGHT 1997 ACS
1988:468156 Document No. 109:68156 Examination of vectors with two dominant, selectable genes for DNA repair and mutation studies in ***mammalian*** cells. Debenham, Paul G.; Webb, Michael B. T.; Stretch, Albert; Thacker, John (Div. Cell Mol. Biol., MRC Radiobiol. Unit, Chilton, OX11 0RD, UK). Mutat. Res., 199(1), 145-58 (English) 1988. CODEN: MUREAV. ISSN: 0027-5107.

AB A series of vectors with 2 dominant selectable genes was constructed for repair and mutation studies following transfer into ***mammalian*** cells. The recombinant genes (SV-gpt and HSVtk-neo) were placed in different relative orientations and positions in the vectors. These variables were shown to affect transformation frequency of cells by the vectors esp. where one of the genes had a relatively weak expression, modelled by truncating the promoter of the HSVtk-neo gene. The use of 2-gene vectors to assess DNA repair was investigated by cutting the SV-gpt ***gene*** with a ***restriction*** ***endonuclease*** and monitoring correct rejoining by selecting for gene activity after transfer into various cell types. In such expts., selection was first applied for the undamaged HSVtk-neo gene to eliminate transfer artifacts, followed by counter-selection of the activity of the damaged SV-gpt gene. The measured frequency of correct rejoining of the damaged gene was found to vary both with the vector construct and with the recipient cell species (Chinese hamster V79 or human transformed fibroblasts). Despite this variation, correct rejoining was found to be consistently lower in radiosensitive (ataxia telangiectasia) human cells than in wild-type human cells, irresp. of the vector construct. In these expts., some of the transformed cell colonies showed sectoring on exposure to the counter-selection, suggesting a slow detn. of the fate of transferred DNA. For mutation studies a V79 cell clone carrying a single copy of one of these 2-gene vectors was identified and shown to be stably integrated. Mutations of the SV-gpt gene in these cells were isolated while maintaining selection of the HSVtk-neo gene, to attempt to limit mutational loss of the total integrated sequence and provide at least one identifiable junction for anal. of deletion events. Spontaneous and X-ray-induced mutants were identified with a variety of genetic changes, as shown by Southern anal., form presumed point mutations to deletions and rearrangements of the vector sequence. Rescue of integrated 2-gene vector sequences from transformed cells, by recloning in Escherichia coli, was shown to be feasible; thus alterations in transferred DNA can be analyzed in detail.

L17 ANSWER 33 OF 46 CAPLUS COPYRIGHT 1997 ACS
1988:217131 Document No. 108:217131 Identification of a second locus encoding .beta.-amylase on chromosome 2 of barley. Kreis, M.; Williamson, M. S.; Shewry, P. R.; Sharp, P.; Gale, M. (Inst. Arable Crop Res., AFRC, Harpenden/Herts., AL5 2JQ, UK). Genet. Res., 51(1), 13-16 (English) 1988. CODEN: GENRA8. ISSN: 0016-6723.
AB A barley endosperm cDNA clone was used to study the polymorphism and chromosomal location of .beta.-amylase genes in barley. Anal. of DNA from 7 cultivars digested with 3 restriction endonucleases showed 2 types of pattern, 1 present in Sultan and the other in the remaining 6 cultivars. A copy-no. reconstruction indicated the presence of .apprx.3 gene copies per haploid genome. Anal. of the 6 available whole chromosome addn. lines and selected telocentric chromosome addns. of barley into wheat showed the location of genes on the short arm of chromosome 2 (probably 1 copy) and the long arm of chromosome 4 (probably 2 copies).

L17 ANSWER 34 OF 46 CAPLUS COPYRIGHT 1997 ACS
1987:569858 Document No. 107:169858 The rat .alpha.-fetoprotein and albumin genes. Transcriptional control and comparison of the sequence organization and promoter region. Nahon, Jean Louis; Danan, Jean Louis; Poiret, Maryse; Tratner, Isabelle; Jose-Estanyol, Matilde; Sala-Trepat, Jose Maria (Lab. Enzymol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, 91190, Fr.). J. Biol. Chem., 262(26), 12479-87 (English) 1987. CODEN: JBCHA3. ISSN: 0021-9258.
AB Functional and structural approaches were used to characterize the transcription units of the rat .alpha.-feto0rotein (AFP) and albumin genes. A cell-free nuclear transcription assay and several genomic clones were used to show that: (1) the rate of transcription of these genes is closely related to the levels of corresponding mRNAs in the yolk sac and those during rat liver development, indicating that the expression of the albumin and AFP genes is mainly regulated

at the transcriptional level in the rat, and (2) the in-vivo 5' end boundaries of the rat AFP and albumin transcription domains were mapped near the resp. first exons. Due to the presence of repeated sequences, the 3'-end boundary of both genes could not be accurately defined in the same manner. (3) No transcription could be detected until 7 kilobases upstream from the cap site of these genes. In addn., the organization of the rat AFP ***gene*** was analyzed by ***restriction*** ***endonuclease*** mapping, S1 nuclease mapping, and nucleotide sequencing. The data show that: (1) the rat AFP gene is 20 kilobase pairs long and is split into 15 exons by 14 intervening sequences; (2) the transcription initiation site of the rat AFP gene is heterogeneous; (3) the 5'-flanking region upstream from the rat AFP gene exhibits 60-90% similarity with the mouse and human AFP genes whereas no major nucleotide identity is found with the rat albumin gene; (4) a 90-base-pair sequence present as one copy upstream from the rat and mouse AFP genes is present as 2 copies in the human genome; (5) several inverted repeats are mapped in the 5'-flaning region indicating potential stem-loop structures. One highly conserved structure encompasses an enhancer-like core sequence and the sequence recognized by the TGGCA-binding protein.

L17 ANSWER 35 OF 46 CAPLUS COPYRIGHT 1997 ACS
1987:453470 Document No. 107:53470 Use of restriction enzymes to detect potential gene sequences in ***mammalian*** DNA. Lindsay, Susan; Bird, Adrian P. (Mamm. Genome Unit, MRC, Edinburgh, EH9 3JT, UK). Nature (London), 327(6120), 336-8 (English) 1987. CODEN: NATUAS. ISSN: 0028-0836.
AB Only a small proportion of the vertebrate genome codes for proteins. It would therefore be useful if genes, and in particular the sites at which transcription begins, could be identified in libraries of cloned DNA. Since many known vertebrate genes have distinctive sequences (HTF-islands) surrounding their transcription start sites, it would be desirable to be able to select these sequences easily and to find out how diagnostic they are for genes. HTF-islands contain a high d. of non-methylated CpG and can be detected in chromosomal DNA as clustered sites for certain rare-cutting (C-G) restriction enzymes. Identification of islands in chromosomal DNA is aided by methylation which blocks C-G enzyme sites in non-island DNA. This advantage is lost in cloned DNA, where CpG methylation is absent. However, even in cloned DNA, most sites for certain C-G enzymes should occur in HTF-islands. This prediction was tested using the enzyme SacII. All 4 sites in sep. cosmids from the human X chromosome were located in HTF-islands. Hybridization to Northern blots provided preliminary evidence that 3 of the islands were assocd. with genes.

L17 ANSWER 36 OF 46 CAPLUS COPYRIGHT 1997 ACS
1986:547159 Document No. 105:147159 Insertion of 1.4 kb and 1.7 kb Mu elements into the Bronze1 gene of Zea mays L. Taylor, L. P.; Chandler, V. L.; Walbot, V. (Dep. Biol. Sci., Stanford Univ., Stanford, CA, 94305, USA). Maydica, 31(1), 31-45 (English) 1986. CODEN: MYDCAH. ISSN: 0025-6153.
AB Two mutable alleles of the Bronze1 locus were recovered from a Robertson's Mutator stock of maize. Genomic restriction mapping demonstrated that both alleles contain Mu element insertions. Gene bz1-mu1 contains a 1.4-kb Mu element, whereas bz1-mu2 contains a 1.7-kb Mu element. The Mu elements inserted within the same transcribed region of the ***gene*** , according to ***restriction*** ***endonuclease*** mapping of genomic DNA.

L17 ANSWER 37 OF 46 CAPLUS COPYRIGHT 1997 ACS
1986:103441 Document No. 104:103441 Mapping a ***mammalian*** mRNA cap site by restriction digestion of hybridized cDNA. Citron, Bruce A.; Darnell, James E., Jr. (Rockefeller Univ., New York, NY, 10021, USA). Gene, 40(1), 131-5 (English) 1985. CODEN: GENED6. ISSN: 0378-1119.
AB A method using restriction enzymes to locate the cap site in a cloned and sequenced 5' region of a gene is described. With a cDNA fragment (or synthetic oligonucleotide sequence), complementary to

any portion of an mRNA (for .alpha.1-antitrypsin [9041-92-3] of mouse), the position of the cap site nucleotide in a genomic clone can be precisely located. The steps include: (1) hybridization of a cDNA fragment to an mRNA sample, and reverse transcription from this primer to produce labeled, fully extended cDNA mols., (2) hybridization of the extended cDNA to a genomic clone contg. the region in which initiation occurs (previously sequenced), (3) restriction endonuclease digestion of the hybrid with .gtoreq.2 enzymes within the putative 1st exon, and (4) size anal. of the short labeled cDNA fragments produced. Multicut restriction enzymes are most useful in this technique. The sizes of the hybridized fragments correspond to the unique distances from the 5' end of the message to each of the different cleavage sites allowing the cap site to be positioned in the genomic sequence.

L17 ANSWER 38 OF 46 CAPLUS COPYRIGHT 1997 ACS
1986:62954 Document No. 104:62954 Principles of production of useful materials by genetic engineering of plants. Ohyama, Kanji; Yamada, Yasuyuki (Fac. Agric., Kyoto Univ., Kyoto, Japan). Hakko to Kogyo, 43(9), 814-20 (Japanese) 1985. CODEN: HAKOD4. ISSN: 0386-0701.
AB A review with 23 refs. on applications of ***plant*** gene manipulation, esp. recombinant DNA techniques, in the prodn. of useful products. ***Gene*** structure, ***restriction*** ***endonuclease*** cleavage of DNA, cloning vectors, and transformation are discussed.

L17 ANSWER 39 OF 46 CAPLUS COPYRIGHT 1997 ACS
1984:623903 Document No. 101:223903 Restriction endonuclease accessibility of the developmentally regulated goat .gamma.-, .beta.C-, and .beta.A-globin genes in chromatin. Differences in 5' regions which show unusually high sequence homology. Liberator, Paul A.; Lingrel, Jerry B. (Coll. Med., Univ. Cincinnati, Cincinnati, OH, 45267-0524, USA). J. Biol. Chem., 259(24), 15497-501 (English) 1984. CODEN: JBCHA3. ISSN: 0021-9258.
AB The chromatin structure of the developmentally regulated fetal (.gamma.), preadult (.beta.c), and adult (.beta.A) .beta.-globin genes of the goat was investigated by using a nuclear restriction enzyme accessibility assay. In fetal liver nuclei, only the PvuII [81295-34-3] site immediately proximal to the .gamma.-globin gene is available for digestion, whereas the homologous recognition sequences 5' to .beta.A and .beta.C are not accessible. Conversely, that site upstream of the .beta.C transcription unit is exclusively digested in bone marrow nuclei prepd. from animals expressing the juvenile form of the protein. In nonerythropoietic tissue, none of the PvuII recognition sequences flanking the 3 genes are digested using identical reaction conditions. These results are particularly striking, since the nucleotide sequence extending for hundreds of bases in either direction from this restriction site is remarkably homologous among the 3 genes. In addn., an endogenous nuclease-hypersensitive site .apprxeq.1150 nucleotides 5' to the .gamma.-globin gene which is evident only in fetal liver tissue was mapped.

L17 ANSWER 40 OF 46 CAPLUS COPYRIGHT 1997 ACS
1984:169250 Document No. 100:169250 Localization of the gene for the P700-chlorophyll a protein in chloroplast DNA from pea and wheat. Smith, Alison G.; Gray, John C. (Bot. Sch., Univ. Cambridge, Cambridge, CB2 3EA, UK). Biochem. Soc. Trans., 12(2), 272-3 (English) 1984. CODEN: BCSTB5. ISSN: 0300-5127.
AB Pea chloroplast DNA, in an Escherichia coli-coupled transcription-translation system, encoded 2 proteins (90,000 and 80,000 mol. wt.) which were pptd. by antibody to P700-chlorophyll a protein. ***Restriction*** ***enzyme*** digestion localized the ***gene*** for these apoproteins to a 2.7-kilobase pair BglII/Cla I fragment at a position near the .alpha.-subunit of ATP synthetase. Similar expts. with cloned restriction fragment wheat chloroplast DNA showed the 2 protein-encoding sequences to be located close to the 3-end of the ATP synthetase .alpha.-subunit gene. It is postulated that the 2 proteins immunoppted. do not

derive from 2 genes, but rather, are precursor forms of the mature 60,000 mol. wt. P700-chlorophyl a protein.

L17 ANSWER 41 OF 46 CAPLUS COPYRIGHT 1997 ACS
1984:1419 Document No. 100:1419 Wheat .alpha.-amylase genes: cloning of a developmentally regulated gene family. Baulcombe, David (Plant Breed. Inst., Trumpington/Cambridge, CB2 2LQ, UK). Genet. Eng., 5, 93-108 (English) 1983. CODEN: GENGDC. ISSN: 0196-3716.
AB DNA complementary to wheat .alpha.-amylase [9000-90-2] mRNA was cloned in plasmid pBR322 and transformed into Escherichia coli. The .alpha.-amylase clones were identified by indirect screening. In distal half grain sections, .alpha.-amylase mRNA is only detectable in the presence of gibberellic acid GA3 [77-06-5]; .alpha.-amylase clones would be included in those clones which hybridize preferentially to poly(A)+ RNA from half grains incubated with GA. Sixty-one .alpha.-amylase-coding clones were identified by this screening method. Use of the isolated gene as a probe to study GA3 regulation of gene expression with respect to development is discussed. Data on the isoenzyme patterns indicate that the .alpha.-amylase gene amy1 may represent a small ***gene*** family and ***restriction*** ***endonuclease*** mapping of 2 cDNA clones suggests that .alpha.-amylase genes may be concd. in the 3' distal third of the mRNA.

L17 ANSWER 42 OF 46 CAPLUS COPYRIGHT 1997 ACS
1983:483212 Document No. 99:83212 A comparison of vertebrate interferon gene families detected by hybridization with human interferon DNA. Wilson, V.; Jeffreys, A. J.; Barrie, P. A.; Boseley, P. G.; Slocombe, P. M.; Easton, A.; Burke, D. C. (Dep. Genet., Univ. Leicester, Leicester, UK). J. Mol. Biol., 166(4), 457-75 (English) 1983. CODEN: JMOBAK. ISSN: 0022-2836.
AB Cloned human interferon cDNAs were used as hybridization probes to detect interferon .alpha. and .beta. ***gene*** families in ***restriction*** ***endonuclease*** digests of total genomic DNA isolated from a wide range of vertebrates and invertebrates. A complex interferon-.alpha. multigene family was detected in all ***mammals*** examd., whereas there was little or no cross-hybridization of human interferon-.alpha. cDNA to nonmammalian vertebrates or invertebrates. In contrast, human interferon-.beta. cDNA detected 1 or 2 interferon-.beta. genes in all ***mammals*** tested, with the exception of the cow and the blackbuck, both of which possessed a complex interferon-.beta. multigene family which has presumably arisen by a recent series of gene duplications. Interferon-.beta. sequences could also be detected in nonmammalian vertebrates which ranged from birds to bony fish. Detailed restriction endonuclease mapping of DNA sequences neighboring the interferon-.beta. gene in a variety of primates indicated a strong evolutionary conservation of flanking sequences, particularly on the 3' side of the gene.

L17 ANSWER 43 OF 46 CAPLUS COPYRIGHT 1997 ACS
1983:138433 Document No. 98:138433 An RNA ***plant*** virus vector or a portion of it, a method for its construction, and a method of producing a gene-derived product from it. Pelcher, Lawrence E.; Halasa, Mary Christine (National Research Council of Canada, Can.). Eur. Pat. Appl. EP 67553 A2 821222, 56 pp. DESIGNATED STATES: R: BE, CH, DE, FR, GB, IT, LI, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 82-302574 820520. PRIORITY: US 81-267539 810527.
AB A viral vector derived from an RNA ***plant*** virus such as tobacco mosaic virus (TMV) can be used to transfer genetic information into whole plants or ***plant*** cells in which the insert-specific proteins can be expressed. The TMV RNA was prepd. as 2 fragments of unequal length. Fragment I comprises the nucleotide sequences which originate from the 5' end of the (+)-strand, extend in the 3' direction, and contain recognition and binding sites for the viral polymerase in the (-)-strand of viral RNA. Fragment II comprises sequences which originate from the 3' end of the (+)-strand, extend in the 5' direction, and contain recognition and binding sites for the viral polymerase in the

(+)-strand. Fragments I or II alone or in combination contain at least a portion of the TMV coat protein gene (CPG), the control region for CPG, and the nucleation region which controls encapsidation by coat protein of the RNA. Three methods are described for viral vector construction with the 2 TMV fragments. In 1 method fragments I and II were mixed together and treated with RNA ligase [37353-39-2] to form the vector RNA (V-RNA). After addn. of coat protein, the vector particles were coinoculated into tobacco along with complete TMV (the complete virus directs synthesis of viral replicase needed for V-RNA replication). The V-RNA was extd. from tobacco using std. methods and reverse-transcribed with reverse transcriptase [9068-38-6] to form a single-stranded DNA copy of V-RNA. DNA polymerase I [9012-90-2] Was used to convert the DNA copy to a double-stranded form, and this was inserted into a bacterial plasmid such as pBR322 to be replicated, selected, and amplified in bacteria. The bacterial DNA plasmid was cleaved in the region corresponding to the coat protein ***gene*** with ***restriction*** ***endonuclease*** [9075-08-5] to allow insertion and ligation of foreign DNA sequences. The recombinant plasmid was allowed to replicate in bacteria. Recombinant RNA contg. V-RNA and sequences corresponding to the foreign gene was transcribed from the plasmid by the bacteria. This recombinant RNA was isolated and encapsidated and was used to inoculate tobacco cells. Since the inserted genetic information, now in the form of RNA, lies within the coat protein gene of V-RNA, the encoded product was produced in large amts. comparable to that of coat protein in complete TMV.
Eukaryotic plants might be superior to bacteria in the cloning and esp. expression of ***eukaryotic*** genes.

L17 ANSWER 44 OF 46 CAPLUS COPYRIGHT 1997 ACS
1978:456315 Document No. 89:56315 Transduction of a bacterial gene into ***mammalian*** cells. Upcroft, P.; Skolnik, H.; Upcroft, J. A.; Solomon, D.; Khoury, G.; Hamer, D. H.; Fareed, G. C. (Mol. Biol. Inst., Univ. California, Los Angeles, Calif., USA). Proc. Natl. Acad. Sci. U. S. A., 75(5), 2117-21 (English) 1978. CODEN: PNASA6. ISSN: 0027-8424.
AB The transduction of an Escherichia coli gene into ***mammalian*** cells is described. A suppressor tRNA gene was linked to a simian virus 40 (SV40) vector in vitro and the recombinant was used to transfect rat embryo cells and monkey kidney cells. The hybrid SV40 genome, SV40-su+III, retained genetic information required for autonomous replication and cellular transformation and had a 1300-base-pair DNA segment in the late ***gene*** region (between the ***restriction*** ***endonuclease*** sites Hpa II at 0.735 and EcoRI at 0/1.0 on the SV40 genetic map) replaced by an 870-base-pair bacterial DNA segment contg. the suppressor tRNA gene, su+III (tRNATyrsu+III). Hybridization with radiolabeled probes specific for vector (SV40) or su+III DNA sequences revealed primarily nonintegrated or free hybrid genomes. In cloned lines of both cell types, the bacterial DNA segment was recovered intact, as judged by the length of the segment excised by restriction endonucleases and its ability to hybridize to the radiolabeled bacterial DNA probe and not to the SV40 probe.

L17 ANSWER 45 OF 46 CAPLUS COPYRIGHT 1997 ACS
1977:168276 Document No. 86:168276 Positions of sea urchin (Strongylocentrotus purpuratus) histone genes relative to restriction endonuclease sites on the chimeric plasmids pSp2 and pSp17. Holmes, David S.; Cohn, Ronald H.; Kedes, Laurence H.; Davidson, Norman (Dep. Chem., California Inst. Technol., Pasadena, Calif., USA). Biochemistry, 16(7), 1504-12 (English) 1977. CODEN: BICHAW.
AB The positions of several sea urchin histone genes on the ***eukaryotic*** fragments of the chimeric plasmids pSp2 and pSp17 were mapped relative to the EcoRI and HindIII restriction endonuclease sites on the plasmids. Two principal mapping methods using the electron microscope were used. There are 2 histone genes, H3 and H2A, on pSp17. There are 2 EcoRI sites at the 2 junctions of

the procaryotic segment with the ***eucaryotic*** segment on the plasmid. For H2A, with a length of 0.52 kbases (kb), 1 end of the gene is situated 0.02-0.03 kb from 1 RI site and there is a HindIII site within this gene at .apprx.0.13 kb from the end proximal to the RI site. The distance from the other RI site to the proximal end of the other gene, H3, is .apprx.0.19 kb. There are 3 histone genes, H2B, H1, and H4, on pSp2. The H2B gene is situated .apprx.0.42 kb from the RI site closest to the procaryotic HindIII site, whereas the H1 gene maps at 0.76 kb from the other RI site of this plasmid. The H4 gene lies between H2B and H1.

L17 ANSWER 46 OF 46 CAPLUS COPYRIGHT 1997 ACS
1975:404816 Document No. 83:4816 Mapping of late adenovirus genes by cell-free translation of RNA selected by hybridization to specific DNA fragments. Lewis, J. B.; Atkins, J. F.; Anderson, C. W.; Baum, P. R.; Gesteland, R. F. (Cold Spring Harbor Lab., Cold Spring Harbor, N. Y., USA). Proc. Natl. Acad. Sci. U. S. A., 72(4), 1344-8 (English) 1975. CODEN: PNASA6.
AB Cytoplasmic RNA, isolated from cells late after infection by adenovirus type 2 and fractionated by hybridization to specific fragments of adenovirus DNA produced by cleavage with the endonuclease R.cntdot.EcoRI, was used as template for protein synthesis in cell-free ***mammalian*** exts. Each of the R.cntdot.EcoRI fragments of DNA selects RNA that encodes specific subsets of the viral polypeptides. From the known order of the R.cntdot.EcoRI fragments, the following partial map is deduced: (III, IIIa, IVa2, V, P-III, IX), (II, P-VI), 100K, IV-where the relative order of the components enclosed in parentheses has not yet been detd.

=> E CHANDRASEGARAN/AU
=> S E4-E6
2 "CHANDRASEGARAN S"/AU
30 "CHANDRASEGARAN SRINIVASAN"/AU
1 "CHANDRASEGARAN SRINIVVASAN"/AU
L18 33 ("CHANDRASEGARAN S"/AU OR "CHANDRASEGARAN SRINIVASAN"/AU OR "CHANDRASEGARAN SRINIVVASAN"/AU)

=> D 1-33 TI

L18 ANSWER 1 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Insertion and deletion mutants of FokI restriction endonuclease

L18 ANSWER 2 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain

L18 ANSWER 3 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Recombinant preparation of hybrid restriction enzyme containing functional domains of Flavobacterium okeanokoities (FokI) restriction endonuclease

L18 ANSWER 4 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Functional domains of FokI restriction endonuclease, hybrid endonucleases containing FokI domains, and recombinant prokaryotes encoding said hybrid enzymes

L18 ANSWER 5 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Functional domains in Flavobacterium okeanokoites restriction endonuclease FokI and nucleic acids encoding these domains

L18 ANSWER 6 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Insertion and deletion mutants of FokI restriction endonuclease

L18 ANSWER 7 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Functional domains of FokI restriction endonuclease, hybrid restriction enzymes containing the FokI recognition domain, and preparation of the domains and enzymes with recombinant prokaryotes

L18 ANSWER 8 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI New vectors for direct cloning of PCR products. [Erratum to document cited in CA120:155096]

L18 ANSWER 9 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Chimeric restriction endonuclease

L18 ANSWER 10 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI New vectors for direct cloning of PCR products

L18 ANSWER 11 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI C-terminal deletion mutants of the FokI restriction endonuclease

L18 ANSWER 12 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Cloning, sequencing, overproduction, and purification of M.cntdot.CviBI (GANTC) methyltransferase from Chlorella virus NC-1A. [Erratum to document cited in CA119(7):66423x]

L18 ANSWER 13 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Cloning, sequencing, overproduction and purification of M CvBI (GANTC) methyltransferase from Chlorella virus NC-1A

L18 ANSWER 14 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Alteration of the cleavage distance of Fok I restriction endonuclease by insertion mutagenesis

L18 ANSWER 15 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Overproduction, purification, and characterization of M.cntdot.HinfI methyltransferase and its deletion mutant. [Erratum to document cited in CA117(1):3096f]

L18 ANSWER 16 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Functional domains in FokI restriction endonuclease

L18 ANSWER 17 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Overproduction, purification and characterization of M.cntdot.HinfI methyltransferase and its deletion mutant

L18 ANSWER 18 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Construction of an efficient overproducer clone of HinfI restriction endonuclease using the polymerase chain reaction

L18 ANSWER 19 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Finding sequence motifs in groups of functionally related proteins

L18 ANSWER 20 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Overproduction and purification of the M.cntdot.HhaII methyltransferase from Haemophilus haemolyticus

L18 ANSWER 21 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Cloning and sequencing the HinfI restriction and modification genes

L18 ANSWER 22 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Helix geometry, hydration, and G.cntdot.A mismatch in a B-DNA decamer

L18 ANSWER 23 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Structural and conformational studies on deoxyguanosyl-3',5'-deoxyadenosine monophosphate and its ethyl phosphotriester analogs - left-handed dimers

L18 ANSWER 24 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Preliminary x-ray diffraction analysis of HhaII endonuclease-DNA cocrystals

L18 ANSWER 25 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Chemical synthesis of oligodeoxyribonucleotides on the polystyrene polymer support

L18 ANSWER 26 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Preparation of three decadeoxyribonucleotides containing an uncommon or modified base

L18 ANSWER 27 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Isolation and purification of deoxyribonucleosides from 90% carbon-13 enriched DNA of algal cells and their characterization by proton and carbon-13 NMR

L18 ANSWER 28 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Phosphorus-31 NMR study of the mechanism of activation and coupling reactions in the synthesis of oligodeoxyribonucleotides by the phosphotriester method

L18 ANSWER 29 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Determination of 3JHF and 4JHF Karplus relationships for the .vphi. and .psi. angles of peptides using N-fluoroamides as models

L18 ANSWER 30 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Synthesis and evaluation of 3-halocyclophosphamides and analogous compounds as novel anticancer "pro-prodrugs"

L18 ANSWER 31 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Detection of a guanine.cntdot.adenine base pair in a decadeoxyribonucleotide by proton magnetic resonance spectroscopy

L18 ANSWER 32 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Investigation of N-fluoroamide derivatives for the determination of the .phi. and .psi. angles of peptide using proton, fluorine-19, and carbon-13 NMR

L18 ANSWER 33 OF 33 CAPLUS COPYRIGHT 1997 ACS
TI Synthesis and template properties of an ethyl phosphotriester modified decadeoxyribonucleotide

=> D 1-11,16 CBIB ABS

L18 ANSWER 1 OF 33 CAPLUS COPYRIGHT 1997 ACS
1996:210069 Document No. 124:254546 Insertion and deletion mutants of FokI restriction endonuclease. ***Chandrasegaran, Srinivasan*** (The Johns Hopkins University, USA). U.S. US 5487994 A 960130, 22 pp. Cont.-in-part of U.S. Ser. No. 126,564. (English). CODEN: USXXAM. APPLICATION: US 94-346293 941123. PRIORITY: US 92-862831 920403; US 93-17493 930212; US 93-126564 930927.
AB The present invention reveals the construction of several insertion (4, 8, 12, 18, 19 or 23 amino acid residues) and deletion (4 or 7 amino acid residues) mutants of the linker region of FokI endonuclease in Flavobacterium okeanokoites. The mutant enzymes were purified, and their cleavage properties were characterized. The mutants have the same DNA sequence-specificity as the wild-type enzyme. However, compared with the wild-type enzyme, the insertion mutants cleave predominantly one nucleotide further away from the recognition site on both strands of the DNA substrate. The four codon deletion mutant shows relaxed specificity at the cut site while the seven codon deletion appears to inactivate the enzyme. The DNA-binding and cleavage domains of FokI appear to be linked by a relatively malleable linker. No simple linear relationship exists between the linker length and the distance of the cut site from the recognition site. Furthermore, the four codon insertion mutants cleave DNA substrates contg. hemi-methylated FokI sites; they do not cleave fully-methylated substrates.

L18 ANSWER 2 OF 33 CAPLUS COPYRIGHT 1997 ACS
1996:93969 Document No. 124:139415 Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Kim, Yang-Gyun; Cha, Jooyeun; ***Chandrasegaran, Srinivasan*** (School Hygiene Public Health, Johns Hopkins University, Baltimore, MD, 21205-2179, USA). Proc. Natl. Acad. Sci. U. S. A., 93(3), 1156-60 (English) 1996. CODEN: PNASA6. ISSN: 0027-8424.

AB A long-term goal in the field of restriction-modification enzymes has been to generate restriction endonucleases with novel sequence specificities by mutating or engineering existing enzymes. This will avoid the increasingly arduous task of extensive screening of bacteria and other microorganisms for new enzymes. Here, the authors report the deliberate creation of novel site-specific endonucleases by linking two different zinc finger proteins to the cleavage domain of Fok I endonuclease. Both fusion proteins are active and under optimal conditions cleave DNA in a sequence-specific manner. Thus, the modular structure of Fok I endonuclease and the zinc finger motifs makes it possible to create "artificial" nucleases that will cut DNA near a predetd. site. This opens the way to generate many new enzymes with tailor-made sequence specificities desirable for various applications.

L18 ANSWER 3 OF 33 CAPLUS COPYRIGHT 1997 ACS
1995:761937 Document No. 123:164077 Recombinant preparation of hybrid restriction enzyme containing functional domains of Flavobacterium okeanokoities (FokI) restriction endonuclease. ***Chandrasegaran,*** *** Srinivasan*** (The Johns Hopkins University, USA). U.S. US 5436150 A 950725, 57 pp. Cont.-in-part of U.S. Ser. No. 17,493, abandoned. (English). CODEN: USXXAM. APPLICATION: US 93-126564 930927. PRIORITY: US 92-862831 920403; US 93-17493 930212.
AB The recognition and cleavage domains of the FokI restriction endonuclease are identified. Accordingly, the present invention relates to DNA segments encoding the recognition and cleavage domains of the FokI restriction endonuclease, resp. The 41 kDa N-terminal fragment constitutes the FokI recognition domain while the 25 kDa C-terminal fragment constitutes the FokI cleavage nuclease domain. The present invention also relates to hybrid restriction enzymes comprising the nuclease domain of the FokI restriction endonuclease linked to a recognition domain of another enzyme. One such hybrid restriction enzyme is Ubx-FN. This enzyme contains the homeo domain of Ubx linked to the cleavage or nuclease domain of FokI. Addnl., the present invention relates to the construction of two insertion mutants of FokI endonuclease.

L18 ANSWER 4 OF 33 CAPLUS COPYRIGHT 1997 ACS
1995:652458 Document No. 123:51090 Functional domains of FokI restriction endonuclease, hybrid endonucleases containing FokI domains, and recombinant prokaryotes encoding said hybrid enzymes. ***Chandrasegaran, Srinivasan*** (Johns-Hopkins University, USA). PCT Int. Appl. WO 9509233 A1 950406, 111 pp. DESIGNATED STATES: W: AU, CA, JP, NZ; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 94-US9143 940823. PRIORITY: US 93-126564 930927.
AB The present invention relates to DNA segments encoding the recognition and cleavage domains of the FokI restriction endonuclease. The 41 kDa N-terminal fragments constitutes the FokI recognition domain while the 25 kDa C-terminal fragment constituents the FokI cleavage nuclease domain. The present invention also relates to hybrid restriction enzymes contg. the nuclease domain of the FokI restriction endonuclease linked to a DNA recognition domain of another protein. One such hybrid restriction enzyme is Ubx-FN. This enzyme contains the homeo domain of Ubx linked to the cleavage or nuclease domain of FokI. Addnl., the present invention relates to the construction of two insertion mutants of FokI endonuclease.

L18 ANSWER 5 OF 33 CAPLUS COPYRIGHT 1997 ACS
1995:227414 Document No. 122:50069 Functional domains in Flavobacterium okeanokoites restriction endonuclease FokI and nucleic acids encoding these domains. ***Chandrasegaran,*** *** Srinivasan*** (The Johns Hopkins University, USA). U.S. US 5356802 A 941018, 23 pp. (English). CODEN: USXXAM. APPLICATION: US 92-862831 920403.
AB The recognition and cleavage domains of the FokI restriction endonuclease have been identified. Accordingly, the present invention relates to DNA segments encoding the recognition and cleavage domains of the FokI restriction endonuclease, resp., and to

the domains themselves. The 41 kDa N-terminal fragment (residues 1-382) constitutes the FokI recognition domain while the 25 kDa C-terminal fragment (residues 383-578) constitutes the FokI cleavage nuclease domain. The present invention also relates to hybrid restriction enzymes comprising the nuclease domain of the FokI restriction endonuclease linked to a recognition domain of another enzyme.

L18 ANSWER 6 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:695939 Document No. 121:295939 Insertion and deletion mutants of FokI restriction endonuclease. Kim, Yang Gyun; Li, Lin; ***Chandrasegaran, Srinivasan*** (Sch. Hygiene and Public Health, Johns Hopkins Univ., Baltimore, MD, 21205-2179, USA). J. Biol. Chem., 269(50), 31978-82 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258.

AB FokI restriction endonuclease recognizes the nonpalindromic pentadeoxyribonucleotide, 5'-GGATG-3':5'-CATCC-3' in duplex DNA and cleaves 9 and 13 nucleotides away from the recognition site. We have reported the presence of two distinct and separable protein domains within this enzyme: one for the sequence-specific recognition of DNA (the DNA binding domain) and the other for the endonucleases activity (the cleavage domain). Our studies have suggested that the two domains are connected by a linker region, which appears to be amenable for repositioning of the DNA-sequence recognition domain with respect to the catalytic domain. Here, we report the construction of several insertion (4-, 8-, 12-, 18-, 19-, or 23-amino acid residues) and deletion (4- or 7-amino acid residues) mutants of the linker region of FokI endonuclease. The mutant enzymes were purified, and their cleavage properties were characterized. The mutants have the same DNA sequence specificity as the wild-type enzyme. However, compared with the wild-type enzyme, the insertion mutants cleave predominantly one nucleotide further away from the recognition site on both strands of the DNA substrate. The four-codon deletion mutant shows relaxed specificity at the cut site while the seven-codon deletion appears to inactivate the enzyme. The DNA binding and cleavage domains of FokI appear to be linked by a relatively malleable linker. No simple linear relationship exists between the linker length and the distance of the cut site from the recognition site. Furthermore, the four-codon insertion mutants cleave DNA substrates contg. hemi-methylated FokI sites; they do not cleave fully methylated substrates. These results are best explained as a consequence of protein-protein interactions between the domains.

L18 ANSWER 7 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:625001 Document No. 121:225001 Functional domains of FokI restriction endonuclease, hybrid restriction enzymes containing the FokI recognition domain, and preparation of the domains and enzymes with recombinant prokaryotes. ***Chandrasegaran, Srinivasan*** (Johns-Hopkins University, USA). PCT Int. Appl. WO 9418313 A1 940818, 105 pp. DESIGNATED STATES: W: AU, CA, JP, NZ; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 94-US1201 940210. PRIORITY: US 93-17493 930212.

AB Proteins contg. only the recognition domain or only the cleavage domain of FokI; hybrid restriction enzymes contg. the FokI cleavage domain fused to another recognition domain; DNA sequences encoding these proteins and DNA constructs contg. the sequences; and prokaryotic cells expressing these constructs are claimed. The 41 kDa N-terminal fragment of FokI was demonstrated to be the recognition domain while the 25 kDa C-terminal fragment was cleavage domain. FokI analogs with altered cleavage patterns were produced with Escherichia coli transformed with plasmids encoding FokI in which the two domains were sepd. by 4 or 7 extra codons.

L18 ANSWER 8 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:500752 Document No. 121:100752 New vectors for direct cloning of PCR products. [Erratum to document cited in CA120:155096]. Cha, Jooyeun; Bishai, William; ***Chandrasegaran, Srinivvasan***

(Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA). Gene, 141(1), 149 (English) 1994. CODEN: GENED6. ISSN: 0378-1119.
AB The errors were not reflected in the abstr. or the index entries.

L18 ANSWER 9 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:186109 Document No. 120:186109 Chimeric restriction endonuclease. Kim, Yang Gyun; ***Chandrasegaran, Srinivasan*** (Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205-2179, USA). Proc. Natl. Acad. Sci. U. S. A., 91(3), 883-7 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.
AB FokI restriction endonuclease recognizes the nonpalindormic pentadeoxyribonucleotide 5'-GGATG-3'.cntdot.5'-CATCC-3' in duplex DNA and cleaves 9 and 13 nt away from the recognition site. Recently, the authors reported the presence of two distinct and separable domains within this enzyme: one for the sequence-specific recognition of DNA (the DNA-binding domain) and the other for the endonuclease activity (the cleavage domain). Here, the authors report the construction of a chimeric restriction endonuclease by linking the Drosophila Ultrabithorax homeodomain to the cleavage domain (FN) of FokI restriction endonuclease. The hybrid enzyme, Ubx-FN, was purified, and its cleavage properties were characterized. The hybrid enzyme shows the same DAN sequence-binding preference as that of Ubx; as expected, it cleaves the DNA away from the recognition site. On the 5'-TTAATGGTT-3' strand the hybrid enzyme cleaves 3 nt away from the recognition site, whereas it cuts the complementary 5'-AACCATTAA-3' strand 8, 9, or 10 nt away from the binding site. Similarly engineered hybrid enzymes could be valuable tools in phys. mapping and sequencing of large eukaryotic genomes.

L18 ANSWER 10 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:155096 Document No. 120:155096 New vectors for direct cloning of PCR products. Cha, Jooyeun; Bishai, William; ***Chandrasegaran,*** *** Srinivasan*** (Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA). Gene, 136(1-2), 369-70 (English) 1993. CODEN: GENED6. ISSN: 0378-1119.
AB The authors describe the construction of two new vectors for direct cloning of polymerase chain reaction (PCR) products. This was done by inserting a synthetic DNA fragment contg. two adjacent XcmI sites between the Asp718 and BamHI sites of the M13mp18 and M13mp19 phages. Cleavage of these M13 derivs. with XcmI will result in a linearized vector with a single thymidine nucleotide at the 3' ends. Thus, these vectors would be very useful for direct cloning of PCR-generated products with high efficiency.

L18 ANSWER 11 OF 33 CAPLUS COPYRIGHT 1997 ACS
1994:2155 Document No. 120:2155 C-terminal deletion mutants of the FokI restriction endonuclease. Li, Lin; Wu, Louisa P.; Clarke, Robert; ***Chandrasegaran, Srinivasan*** (Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA). Gene, 133(1), 79-84 (English) 1993. CODEN: GENED6. ISSN: 0378-1119.
AB The authors have constructed two C-terminal deletion mutants of the FokI restriction endonuclease by using the polymerase-chain-reaction technique and expressed them in Escherichia coli. The two mutant proteins (MP) of 41 and 30 kDa, were purified to homogeneity and their DNA-binding properties were characterized. The 41-kDa MP specifically binds the DNA sequence, 5'-GGATG 3'-CCTAC, like the wild-type (wt) FokI, but does not cleave DNA. The 30-kDa MP does not bind DNA. The affinity of the 4-kDa MP for the DNA substrate is comparable to that of wild type FokI. The 41-kDa MP interacts with its substrate like the wild type FokI, as revealed by hydroxyl radical footprinting expts. In the presence of a DNA substrate, the 4-kDa MP is cleaved by trypsin into a 30-kDa N-terminal fragment and an 11-kDa C-terminal fragment. Addn. of the HPLC-purified 11-kDa C-terminal fragment to the 30-kDa MP restores its sequence-specific DNA binding property. These results confirm that the N-terminal 41-kDa fragment of the FokI endonuclease constitutes the DNA recognition domain of the endonuclease.

L18 ANSWER 16 OF 33 CAPLUS COPYRIGHT 1997 ACS
1992:403270 Document No. 117:3270 Functional domains in FokI restriction endonuclease. Li, Lin; Wu, Louisa P.; ***Chandrasegaran, Srinivasan*** (Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205-2179, USA). Proc. Natl. Acad. Sci. U. S. A., 89(10), 4275-9 (English) 1992. CODEN: PNASA6. ISSN: 0027-8424.

AB The polymerase chain reaction was used to alter transcriptional and translational signals surrounding the Flavobacterium okeanokoites restriction endonuclease (fokIR) gene, so as to achieve high expression in Escherichia coli. By changing the ribosome-binding site sequence preceding the fokIR gene to match the consensus E. coli signal and by placing a pos. retroregulator stem-loop sequence downstream of the gene, FokI yield was increased to 5-8% of total cellular protein. FokI was purified to homogeneity with phosphocellulose, DEAE-Sephadex, and gel chromatog., yielding 50 mg of pure FokI endonuclease per L of culture medium. The recognition and cleavage domains of FokI were analyzed by trypsin digestion. FokI, in the absence of a DNA substrate, cleaved into a 58-kDa C-terminal and an 8-kDa N-terminal fragment. The 58-kDa fragment did not bind the DNA substrate. FokI, in the presence of a DNA substrate, cleaved into a 41-kDa N-terminal fragment and a 25-kDa C-terminal fragment. On further digestion, the 41-kDa fragment degraded into 30-kDa N-terminal and 11-kDa C-terminal fragments. The cleaved fragments both bound DNA substrates, as did the 41-kDa fragment. Gel mobility-shift assays indicated that all of the protein contacts necessary for the sequence-specific recognition of DNA substrates were encoded within the 41-kDa fragment. Thus, the 41-kDa N-terminal fragment constitutes the FokI recognition domain. The 25-kDa fragment, purified by DEAE-Sephadex column chromatog., cleaved nonspecifically both methylated (pACYCfokIM) and nonmethylated (pTZ19R) DNA substrates in the presence of MgCl2. Thus, the 25-kDa C-terminal fragment constitutes the FokI cleavage domain.

```
FILE 'USPAT' ENTERED AT 14:25:43 ON 01 MAY 1997

=> S RESTRICTION(W)(ENDONUCLEASE OR ENZYME)
         59150 RESTRICTION
          3575 ENDONUCLEASE
         42023 ENZYME
L1        5337 RESTRICTION(W)(ENDONUCLEASE OR ENZYME)

=> S GENE;S L1 AND L2
L2       12402 GENE


L3        5050 L1 AND L2

=> S MAMMAL?;S PLANT;S EUKARYOT? OR EUCARYOT?
L4       37427 MAMMAL?


L5      108223 PLANT


          4189 EUKARYOT?
          1400 EUCARYOT?
L6        5205 EUKARYOT? OR EUCARYOT?

=> S L3 AND L4;S L3 AND L5;S L3 AND L6
L7        2653 L3 AND L4


L8        1254 L3 AND L5


L9        2724 L3 AND L6

=> S ZF(4A)FN
           667 ZF
          4818 FN
L10          0 ZF(4A)FN

=> E CHANDRASEGARAN/IN
=> S E4
L11          3 "CHANDRASEGARAN, SRINIVASAN"/IN

=> D 1-3

1.  5,487,994, Jan. 30, 1996, Insertion and deletion mutants of FokI
restriction endonuclease; Srinivasan Chandrasegaran, 435/199, 193 [IMAGE
AVAILABLE]

2.  5,436,150, Jul. 25, 1995, Functional domains in flavobacterium
okeanokoities (foki) restriction endonuclease; Srinivasan Chandrasegaran,
435/199, 69.7, 252.33; 536/23.2 [IMAGE AVAILABLE]

3.  5,356,802, Oct. 18, 1994, Functional domains in flavobacterium
okeanokoites (FokI) restriction endonuclease; Srinivasan Chandrasegaran,
435/199; 536/23.2, 23.4 [IMAGE AVAILABLE]
```